EFFECT OF TEMPERATURE ON PHASE VARIATION

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A monophasic variant of Sal. typhimurium SW1061 is motile in phase-2 but non-motile in phase-1. This is greatly advantageous for the quantitative studies of phase variation, because each of the alternative phases can be scored on a NGA plate without the help of antiserum; a phase-1 clone grows into a colony whereas a phase-2 clone grows into a swarm. An application of the technic to the test of temperature effect on phase variation will be reported here.

I. EFFECT OF TEMPERATURE ON THE PHASE-EQUILIBRIUM IN GROWING CULTURE OF SW1061.

Methods.

A penassay broth culture of SW1061 is plated with NGA. A swarm and a colony are isolated from it into penassay broth media. After 4hours' culture at 37C, the two cultures are mixed and inoculated into 3 penassay broth media (0.1 ml to 10 ml medium). They are cultured at 15C, 28C or 37C, one culture for each temperature. 37C and 28C cultures are subcultured daily and 15C culture is subcultured weekly. 2 weeks after the initiation of the cultures, 37C culture is devided into three, and one is continued to grow at 37C, and the other two are at 28C and 15C respectively. On the course of subculture , a sample of each culture is diluted and plated with NGA medium. NGA plates are kept at room temperature and numbers of colony (phase-1) and swarms (phase-2) are scored after 15 to 20 hours.

Results.

The results were summarized in table 1.

As a whole, the 15C culture maintains the value between 50 and 70 C(phase-1)%. The 28C culture tends to increase up to 70-80 C%, whereas the 37C culture decreases below 50%. The process is complicated by the intermix of decrease and increase, presumably by the operation of periodic selection.

When the 37C culture was transferred to 27C after 2 weeks' culture, the frequency became close to that of the culture which has been maintained at 27C from the beginning. The same is also true when the 37C culture was transferred to 15C. Consequently, the difference in the equilibrium may be not simply caused by a secondary change of the cultures after the initiation of the experiment. The experiment to test the repeatability of the results is on the way.

Table 1.

Effect of temperature on the phase-equilibrium in growing culture of SW1061.

Day	Culture at 15C				Culture at 28C				Culture at 37C			
	C	S	T	C%	C	S	T	C%	C	S	T	C%
0	103	62	165	63.4	103	62	165	63.4	103	62	165	63.4
1	739	298	1037	71.3	1016	427	1443	70.4	215	104	319	67.4
2	248	140	388	63.9	169	66	235	71.9	200	244	444	45.0
5	226	147	373	60.6	292	107	399	73.2	66	93	159	41.5
6	126	81	207	60.9	640	298	938	63.2	262	357	619	42.3
7	280	197	487	58.7	193	71	264	73.1	324	595	919	35.3
8	30	30	60	50.0	403	110	513	78.8	81	189	270	30.0
9	360	301	661	54.5	160	53	213	75.1	137	452	589	23.3
11	153	131	284	53.9	68	101	169	40.2	179	386	565	31.7
14	283	282	565	50.1	244	188	432	56.4	138	107	245	56.5
22	665	273	938	70.9	357	86	443	80.6	11	15	26	42.3
22	* 85	49	134	63.5	* 188	47	235	80.0				

C:colony (phase-1), S:swarm (phase-2), T:total

* transferred from 37C culture at 14th day.

II. EFFECT OF SUBLETHAL DOSE OF TEMPERATURE ON THE EXPRESSION OF PHASE IN SAL. TYPHIMURIUM.

Methods.

The experimental procedures are as follows:

1). Streak SW1061 penassay broth culture onto an EMB-gal plate.

2). Isolate a colony of non-motile phase to penassay broth medium (10 ml).

3). Culture on rotator at 37C, 4 hours. Devide into three parts.

4). (a) Dilute and plate on NGA media.

(b) Keep 30 minutes at 37C; then dilute and plate on NGA.

(c) Keep 30 minutes in 50C water bath; then dilute and plate on NGA.

On NGA media, phase-1 cells grow into colonies and phase-2 cells into swarms. For the experiment on TM2, anti-1,2 NGA plates were used instead of plain NGA. Consequently, phase-1 appears as swarm and phase-2 as colony. It is possible that the increase of a number of colony is caused not by phase variation but by mutation to Fla^-. The possibility was examined in a control experiment in which TM2 was plated not on anti-1,2 NGA but on plain NGA.

Results.

The results are summarized in table 1. SW1061 tends to increase the frequency of Fla^- by a high temperature treatment. The increase is not significant enough statistically in the experiment 1 (see X^2 test). The experiments 2 and 3 show the increase with statistical significance. The control (TM2 on NGA plates) did not produce any Fla^- colony from a sample tested. Therefore, the increase of Fla^- in SW1061 may be accounted for by the change of phase-2 to phase-1. The fraction (a) of phase-2 cells which changed to phase-1 is calculated from the following formulae:

$$a = \frac{p'-p}{1-p} \quad \text{or} \quad a = \frac{q-q'}{q}.$$

p: frequency of phase-1 at time 0.
p': " " " after treatment.
q: frequency of phase-2 at time 0.
q': " " " after treatment.

The calculated values are, $a_1 = 0.3$, $a_2 = 0.1$, & $a_3 = 0.03$.

The experiment on TM2, however, does not support above conclusion; that is, significant increase in phase-1 is not shown in this case (experiment 5.2 must produce more than 10 swarms, if the change from phase-2 to 1 occurred at about

the same frequency as calculate from the data on SW1061).

In conclusion, a series of experiments on SW1061 suggests that high temperature treatment induces a change from phase-2 to phase-1 (inactivation of H_2). The generality of the effect, however, is still in question.

Table 1

Effect of sublethal dose of temperature on the expression of phase in Sal. typhimurium SW1061 Fla⁻:1,2 and TM2 i:1,2.

SW1061

Expt. Number	Treatment Time (min.)	Temp. (C)	Number of Survival cells (per ml)	Number of Colonies (Fla⁻)	Swarms (1,2)	Total	Frequency of swarm
1.1	0	/	8.5×10^8	6386	16	6852	2.3×10^{-3}
".2	30	37	3.2×10^9	7194	21	7215	2.9×10^{-3}
".3	30	50	4.7×10^7	7275	11	7286	1.5×10^{-3}
2.1	0	/	7.7×10^6	1064	197	1261	0.156
".2	30	37	1.7×10^7	1307	245	1552	0.158
".3	30	50	7.4×10^5	3219	494	3713	0.133
							Frequency of colony
3.1	0	/	6.7×10^8	20	423	443	4.5×10^{-2}
".2	30	37	7.7×10^8	22	467	489	4.5×10^{-2}
".3	30	50	1.5×10^8	60	717	777	7.7×10^{-2}
Control: TM2 i:1,2				(Fla⁻ mutant)	(phase-1 & -2)		
4.1	0	/	9.4×10^8	0	561	561	0
".2	30	37	3.1×10^9	0	305	305	0
".3	30	50	1.8×10^7	0	854	854	0
TM2 (on anti-1,2 NGA plate)				(phase-2)	(phase-1)		
5.1	0	/	1.1×10^9	591	1	592	1.7×10^{-3}
".2	30	50	4.1×10^7	508	1	509	2.0×10^{-3}

		X^2	P between
X^2 test,	1.1 & 1.3	1.3	0.2 and 0.3
	1.2 & 1.3	3.2	0.05 and 0.1
	2.1 & 2.3	4.3	0.02 and 0.05
	2.2 & 2.3	5.6	0.01 and 0.02
	3.1 & 3.3	4.7	0.02 and 0.05
	3.2 & 3.3	5.2	0.02 and 0.05